Pentachlorophenol and Hydroxylated Polychlorinated Biphenyl Metabolites in Umbilical Cord Plasma of Neonates from Coastal Populations in Québec

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Concentrations of polychlorinated biphenyls (PCBs), hydroxylated metabolites of PCBs (HO-PCBs) and octachlorostyrene (4-HO-HpCS), and pentachlorophenol (PCP) were determined in umbilical cord plasma samples from three different regions of Québec. The regions studied included two coastal areas where exposure to PCBs is high because of marine-food–based diets—Nunavik (Inuit people) and the Lower North Shore of the Gulf of St. Lawrence (subsistence fishermen)—and a southern Québec urban center where PCB exposure is at background levels (Québec City). The main chlorinated phenolic compound in all regions was PCP. Concentrations of PCP were not significantly different among regions (geometric mean concentration 1,670 pg/g, range 628–7,680 pg/g wet weight in plasma). The ratio of PCP to polychlorinated biphenyl congener number 153 (CB153) concentration ranged from 0.72 to 42.3. Sum HO-PCB (ΣHO-PCBs) concentrations were different among regions, with geometric mean concentrations of 553 (range 238–1,750), 286 (103–788), and 234 (147–464) pg/g wet weight plasma for the Lower North Shore, Nunavik, and the southern Québec groups, respectively. Lower North Shore samples also had the highest geometric mean concentration of sum PCBs (sum of 49 congeners; ΣPCBs), 2,710 (525–7,720) pg/g wet weight plasma. ΣPCB concentrations for Nunavik samples and southern samples were 1,510 (309–6,230) and 843 (290–1,650) pg/g wet weight plasma. Concentrations (log transformed) of ΣHO-PCBs and ΣPCBs were significantly correlated ($r = 0.62$, $p < 0.001$), as were concentrations of all major individual HO-PCB congeners and individual PCB congeners. In Nunavik and Lower North Shore samples, free thyroxine (T_4) concentrations (log transformed) were negatively correlated with the sum of quantitated chlorinated phenolic compounds (sum PCP and ΣHO-PCBs; $r = –0.47$, $p = 0.01$, $n = 20$) and were not correlated with any PCB congeners or ΣPCBs. This suggests that PCP and HO-PCBs are possibly altering thyroid hormone status in newborns, which could lead to neurodevelopmental effects in infants. Further studies are needed to examine the effects of chlorinated phenolic compounds on thyroid hormone status in newborns. *Key words:* **hydroxylated metabolites, pentachlorophenol, polychlorinated biphenyls, retinol, thyroxine, umbilical cord plasma.** *Environ Health Perspect* **110:411–417 (2002). [Online 12 March 2002]**
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Polychlorinated biphenyls (PCBs) have been well studied for possible effects on newborns and infants after it was determined that PCBs could effectively pass through the placental barrier and that they were associated with lower birth weights (*1*). Jacobson et al. (*2*) found that children exposed *in utero* to PCBs had delayed central nervous system functioning. Subsequent studies then confirmed that for this same cohort, reductions in cognitive function were associated with higher *in utero* PCB exposure at 4 years of age (*3*), followed by lower IQs at 11 years of age (*4*). These studies seem to indicate a potential link between PCBs and neurodevelopment.

Although many theories exist about how PCBs affect neurodevelopment, the main hypothesis involves disruption of thyroid hormone homeostasis (*5*). Thyroid hormones regulate neuronal proliferation and cell migration and differentiation, including control over when differentiation begins and when cell proliferation ends (*6*). Studies in the rat showed that transport of thyroid hormones to the brain requires thyroxine (T_4) to pass through the blood–brain barrier bound to the thyroid hormone transport protein transthyretin (TTR) (*7*). Although PCBs show some binding affinity for TTR (*8*), hydroxylated metabolites of PCBs (HO-PCBs) have much higher *in vitro* binding affinities that can be as high as 12 times the binding affinity of the natural ligand T_4 (*9*). Binding to TTR is not limited to HO-PCBs. Other halogenated phenolic compounds such as pentachlorophenol (PCP), halogenated phenols, and tetrabromobisphenol A (*10,11*) also have strong affinities for TTR. Recently, PCP was found to be the dominant phenolic compound in whole blood from Inuit (*12*) and Latvian and Swedish fish eaters (*13*). Thus, we must consider the combined effect of halogenated phenolic compounds in plasma that exhibit similar toxicological properties to HO-PCBs (*10,14,15*). A recent review described the formation and retention of HO-PCBs and the main metabolites that have been previously identified in plasma (*16*).

HO-PCBs decrease circulating levels of thyroid hormones in rats through competitive binding to TTR (*17*). TTR is also responsible for retinol transport by forming a dimer with retinol-binding protein. Thus, circulating retinol concentrations can also be affected by PCB and HO-PCB exposure (*18*).

The fetus may be especially vulnerable to PCB and HO-PCB exposure. When fetal mice were exposed *in utero* to 4′-HO-CB79, a metabolite of polychlorinated biphenyl congener number 77 (CB77), both maternal and fetal plasma T_4 levels decreased significantly compared to controls (*19*). In this same study, fetal plasma had twice the 4′-HO-CB79 concentration of the maternal plasma (*20*). These experiments were recently repeated on pregnant rats orally exposed to 4-HO-CB107 (*21*), one of the main HO-PCBs found in human plasma (*12,13*). In that study, both maternal and fetal plasma concentrations of thyroid hormones were reduced by exposure to 4-HO-CB107. Fetal total T_4 concentrations decreased by 89% of that of the controls (*21*). The decreased plasma T_4 levels also decreased forebrain and cerebellum T_4 concentrations compared to controls (*21*), which may lead to a neurodevelopmental effect. PCP also decreases brain T_4 availability in rats (*22*). Another interesting finding of the 4-HO-CB107 rat dosing study was

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an accumulation of 4-HO-CB107 in fetal plasma, liver, and brain (*21*).

Thus, prenatal exposure to PCBs, HO-PCBs, and PCP may all lead to thyroid hormone disruption and possibly neurodevelopmental effects. Analysis of umbilical cord plasma is of special interest because it provides a direct indication of *in utero* exposure to developmental toxicants. PCBs have been measured previously in umbilical cord plasma (*23,24*), but the present study, to our knowledge, is one of the first studies to examine chlorinated phenolic compounds in this biological medium. Participants were from populations with different PCB exposures caused by differences in dietary habits. Retinol and thyroid hormone status [triiodothyronine (T_3), free T_4, thyroid-stimulating hormone (TSH), and thyroxine-binding globulin (TBG)] were determined in samples from remote maritime populations, so the relationship between chlorinated phenolic compounds and these biological markers could be explored.

Materials and Methods

Samples. Plasma samples were obtained during various umbilical cord blood surveys conducted from 1993 to 1996 in Québec (*25,26*). These surveys took place in Nunavik (northern Québec), the Lower North Shore of the Gulf of St. Lawrence, and southern Québec (Québec City; Figure 1). The population in the Québec City area receives background PCB exposure similar to that of the general population of Canada, whereas the former two coastal areas comprise small settlements of people with unusually high PCB exposure. The traditional diet of Nunavik Inuit include seal and beluga blubber, which contain concentrations of PCBs in the order of several milligrams per kilogram (*27,28*). The diet of the Lower North Shore subsistence fish-eating population includes fish, sea mammals, and seabird eggs (*28*). Ten samples from each region were randomly selected for chlorinated phenolic compound and PCB residue analysis from all samples collected during the surveys. Nunavik samples were all from Inuit newborns, southern Québec samples from Caucasian newborns, and Lower North Shore samples from three Caucasians and seven aboriginal neonates.

Standards and chemicals. PCBs are numbered according to the numbering scheme as described by Ballschmiter and Zell (*29*). Hydroxylated PCBs and their methoxylated derivatives are given the appropriate Ballschmiter PCB number according to their chlorination pattern. The HO- or MeO-functional groups are numbered thereafter, as described by Letcher et al. (*16*). Note that the numbering of two congeners in our previous publication (*12*) has changed: 4-HO-CB109 is now 4-HO-CB107, and 4-HO-CB107 is now 4′-HO-CB108.

The following $^{13}C_{12}$-labeled standards were acquired from Wellington Laboratories (Guelph, ON, Canada) and were used as an internal recovery standard mixture: 4′-HO-CB120, 4′-HO-CB159, 4′-HO-CB172, and 4-HO-CB187. [$^{13}C_6$]PCP was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and was used for PCP quantitation. Labeled PCBs ([$^{13}C_{12}$]CB-118, 153, 180, and 194) were used as internal recovery standards, and [$^{13}C_{12}$]CB-138 was used as the performance standard for PCB analysis. [$^{13}C_{12}$]PCB standards

Figure 1. Sample locations for the three populations: Nunavik (northern Québec), Lower North Shore (Gulf of St. Lawrence), and southern Québec (Québec City).

Table 1. Concentrations (picograms per gram wet weight plasma) of halogenated phenolic compounds and ΣPCBs in umbilical cord plasma from three regions in Québec (n = 10 for each region).

Compound	Nunavik			Lower North Shore			Southern Québec		
	GM	Min	Max	GM	Min	Max	GM	Min	Max
PCP	1,870	889	7,680	1,430	628	3,640	1,740	1,020	4,090
4-HO-HpCS	31	3	177	34	9	139	5	2	21
HO-PCBs									
4-HO-CB187	47	13	155	95	54	250	28	10	97
4-HO-CB146	37	4	134	81	16	507	12	4	58
3-HO-CB153	19	4	65	23	10	74	6	3	14
4-HO-CB107/ 4′-HO-CB108	12	3	44	49	6	168	11	3	43
3′-HO-CB138	10	3	35	22	9	92	5	3	16
4′-HO-CB172	10	3	43	20	8	75	4	1	11
4,4′-diHO-CB202[a]	6	3	15	5	1	13	4	3	17
3-HO-CB187	4	ND	34	7	2	21	1	ND	3
4-HO-CB193	3	1	17	3	1	8	1	0	5
4′-HO-CB120	2	1	4	7	3	20	2	ND	6
4′-HO-CB208[a]	2	1	5	3	1	11	1	ND	3
3′-HO-CB180	2	ND	14	5	1	23	1	ND	3
4′-HO-CB130	1	ND	3	2	ND	27	1	ND	3
4′-HO-CB199[a]	< 1	ND	4	< 1	ND	7	< 1	ND	< 1
Sum HO-PCBs	286	103	788	553	238	1,750	234	147	464
HO-PCBs:PCBs	0.19	0.08	0.41	0.20	0.08	0.56	0.19	0.04	0.46

Abbreviations: GM, geometric mean; Max, maximum; Min, minimum; ND, not detected.
[a]Tentative identifications based on information in the review by Letcher et al. (*16*). Note that 4-HO-CB107 and 4′-HO-CB108 coelute and were quantitated as a single peak. The 4-hydroxy-heptachlorostyrene (4-HO-HpCS) was quantitated using relative response factors from the heptachlorinated MeO-PCB standards.

were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The HO-PCB performance standard, 4′-Me-4-MeO-CB112, was a custom synthesis by B. Wightman (Carleton University, Ottawa, ON, Canada).

All solvents were residue-analysis grade and purchased from EM Science (Gibbstown, NJ, USA). Merck Silica gel (Grade 60, 70-230 mesh, 60Å) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Sulfuric acid (trace-metal grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA).

Methodology and instrumentation. A thorough description of the methodology and instrumentation used for these analyses was described previously (*12*). Methodology was altered slightly for this study. Umbilical cord plasma samples ranged from 1.63 to 10.4 g and samples were spiked with 20 µL [$^{13}C_{12}$]HO-PCB internal standard mixture (100 pg/µL), 20 µL [$^{13}C_6$]PCP (100 pg/µL), and with 10 µL [$^{13}C_{12}$]PCB internal standard mixture (2.5 ng/µL) before extraction. The final volume for the phenolic compound fraction was 25 µL and was spiked with 4′-Me-4-MeO-2,3,3′,5,6-pentachlorobiphenyl (5 µL) as performance standard before analysis. The PCB fraction was reduced to a final volume of 100 µL and spiked with [$^{13}C_{12}$]CB138 performance standard (10 µL) before analysis. Because of low levels of PCBs in the umbilical cord plasma samples, we analyzed PCBs by GC-MS electron capture negative chemical ionization mode using the same mass spectrometry conditions as described previously for HO-PCBs (*12*). Only pentachlorinated PCB congeners and higher are reported because tetrachlorinated congeners and lower do not respond well to this type of detection. Congener-specific analysis using a characterized Aroclor 1:1:1 quantitation mixture allowed the quantitation of 49 PCB congeners in most of the samples.

Retinol analysis was performed at the Québec Toxicology Centre (Sainte-Foy, Québec, Canada). Ethanol was added to the plasma sample to denature proteins, and retinol was extracted from the resulting solution with hexane. The hexane extract was concentrated under vacuum (Speed-Vac) and redissolved in ethanol. Retinol was determined by reverse-phase high-pressure liquid chromatography (Waters Corp., Milford, MA, USA) using a C-18 column and a UV detector (325 nm). Free T_4, T_3, TSH, and TBG were measured by heterogeneous competition magnetic separation assay (Immuno 1 System; Bayer Diagnostics, Leverkusen, Germany), and TBG was determined by radioimmunoassay (DiaSorin, Stillwater, MN, USA). Thyroid hormones and TBG determinations were conducted at the Unité de Recherche en Génétique Humaine (CHUL-CHUQ, Sainte-Foy, Québec, Canada). Thyroid hormone measurements were performed on Nunavik and Lower North Shore samples but not on southern Québec samples.

All statistical analyses were completed with STATISTICA for Windows, version 5.1, from StatSoft, Inc. (Tulsa, OK, USA).

Table 2. Concentrations (picograms per gram wet weight plasma) of 49 PCB congeners in umbilical cord plasma from three regions in Québec (n = 10 for each region).

	Nunavik			Lower North Shore			Southern Québec		
Congener	GM	Min	Max	GM	Min	Max	GM	Min	Max
CB92	9	ND	60	7	ND	167	9	ND	13
CB84	27	5	141	36	6	251	16	ND	31
CB101/90	49	11	262	87	13	786	44	17	181
CB99	100	16	1,120	174	17	1,630	38	ND	116
CB97	10	ND	167	12	ND	232	8	ND	19
CB87	28	5	236	63	8	436	13	ND	27
CB85	6	2	115	14	2	150	6	ND	86
CB110	42	8	403	79	10	709	44	18	502
CB118	67	19	402	155	30	673	35	9	81
CB105	19	6	300	37	7	155	11	2	31
CB136	13	1	316	15	2	651	12	1	536
CB151	7	3	34	11	4	68	8	ND	14
CB144/135	17	ND	97	27	ND	713	13	ND	22
CB149	20	9	71	33	ND	96	30	14	103
CB134	8	1	35	11	2	86	3	ND	18
CB146	23	5	98	54	15	178	11	6	54
CB153	262	49	1,340	430	107	1,350	104	30	199
CB141	3	2	20	5	3	13	5	1	8
CB130	6	2	20	9	3	30	2	ND	4
CB137	4	1	13	6	2	15	2	ND	3
CB138/163	157	36	712	232	62	704	54	11	110
CB158	5	2	18	8	2	17	3	1	6
CB178	1	ND	9	1	ND	3	1	ND	1
CB128	7	3	27	16	6	47	4	ND	7
CB156	27	5	94	40	17	104	11	2	19
CB157	8	2	28	17	7	45	5	ND	8
CB179	2	1	5	2	ND	4	2	ND	5
CB176	1	ND	2	1	ND	1	1	ND	2
CB178	2	< 1	27	1	< 1	20	1	ND	4
CB187/182	39	7	146	102	24	297	38	13	226
CB183	14	4	135	23	6	57	7	2	12
CB185	< 1	< 1	2	1	ND	1	1	ND	2
CB174	3	2	12	4	2	9	6	1	11
CB177	5	2	13	10	4	18	4	1	7
CB171	3	1	7	6	2	13	2	1	4
CB172	2	< 1	10	6	2	14	1	ND	3
CB180	118	33	663	146	43	501	40	8	84
CB193	3	< 1	53	5	1	23	1	< 1	4
CB191	< 1	< 1	5	1	< 1	7	1	< 1	2
CB170/190	21	4	74	39	13	87	13	3	22
CB202	4	1	21	5	3	10	2	ND	3
CB200	1	< 1	22	2	1	4	1	ND	2
CB199	1	ND	4	1	ND	4	2	ND	6
CB201	4	1	17	10	2	31	7	4	11
CB196/203	8	2	26	33	7	113	15	6	95
CB195	3	1	28	5	1	32	2	ND	2
CB194	9	2	23	17	7	55	11	2	21
CB206	3	1	10	6	3	12	1	ND	2
CB209	1	< 1	1	1	< 1	2	< 1	ND	1
Sum PCBs	1,510	309	6,230	2,710	525	7,720	843	290	1,650

Abbreviations: GM, geometric mean; Max, maximum; Min, minimum; ND, not detected.

Table 3. Concentrations of retinol, thyroid hormones, and TBG in umbilical cord plasma samples from three regions in Québec (n = 10 for each region).

	Nunavik			Lower North Shore			Southern Québec		
	GM	Min	Max	GM	Min	Max	GM	Min	Max
Retinol (µg/L)	160	61	250	160	89	290	190	110	330
FT_4 (pmol/L)	16	13	22	17	9.6	21	NA	NA	NA
T_3 (nmol/L)	0.64	0.45	1.20	0.49	0.20	0.78	NA	NA	NA
TSH (µmol/L)	7.7	3.9	19	6.7	3.9	15	NA	NA	NA
TBG (nmol/L)	920	590	1,300	880	620	1,300	NA	NA	NA

Abbreviations: GM, geometric mean; Max, maximum; Min, minimum; NA, not analyzed.

Results

Recoveries of the internal recovery standards ([$^{13}C_6$]PCP and [$^{13}C_{12}$]HO-PCBs and PCBs) were in the range of 75–104%. Mean recovery of phenolic internal standards was better than 87%. All concentrations were recovery corrected.

With the Liliefors test for normal distribution, the chemical residue data were not normally distributed. Thus, all data (including retinol and thyroid hormone concentrations) were log transformed before statistical analysis. The regional concentration data are summarized using geometric means along with minimum and maximum values (Tables 1–3)

Thirty compounds were characterized as HO-PCBs in the umbilical cord plasma samples. Concentrations of PCP and identified HO-PCB congeners are listed in Table 1. Two congeners, 4-HO-CB107 and 4′-HO-CB108, coelute and were quantitated as a single peak. The peak is likely 4-HO-CB107, as demonstrated in previous studies (*16,30*). ΣHO-PCBs represents a sum of all identified HO-PCBs and all compounds characterized as HO-PCBs. Unidentified HO-PCBs were quantitated using relative response factors as described previously (*12*). ΣHO-PCBs were analyzed for regional differences by multiple analysis of variance. Lower North Shore samples had the highest mean concentration of ΣHO-PCBs, which was significantly higher than concentrations in southern Québec samples using the Sheffe test ($p = 0.01$). The Nunavik samples were not significantly different from southern samples ($p = 0.8$) or from Lower North Shore samples ($p = 0.06$). PCP concentrations were highest in Nunavik samples but were not significantly different among regions.

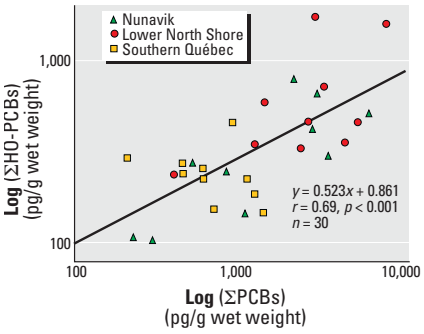

Figure 2. Relationship between log-transformed ΣHO-PCBs and ΣPCBs concentrations.

We also found another compound recently identified as a major chlorinated phenolic compound in polar bear plasma, 4-hydroxy-heptachlorostyrene (4-HO-HpCS) (*31*), in the human umbilical cord plasma samples (Table 1). This compound was determined in all umbilical cord plasma samples. No quantitative standard was available at the time of analysis, so we estimated concentrations of 4-HO-HpCS using the average heptachlorinated MeO-PCB response factor. The geometric mean concentrations in Nunavik and Lower North Shore samples were about six times higher than in southern Québec samples.

Forty-nine PCB congeners with 5 or more chlorines were above the detection limit in most of the umbilical cord plasma samples. Concentrations of all 49 PCBs and ΣPCBs (sum of all congeners) are listed in Table 2. The ratio of ΣHO-PCBs to ΣPCBs is given in Table 1. ΣPCBs were highest in Lower North Shore plasma samples and Nunavik samples, but only Lower North Shore samples were significantly different ($p = 0.01$) from southern Québec samples by the Sheffe test. The mean ratio of ΣHO-PCB metabolites to PCBs was highest in southern Québec samples and lowest in Nunavik samples, but the ratio was not statistically different among regions.

ΣHO-PCBs and ΣPCBs concentrations were highly correlated in umbilical cord plasma ($r = 0.69$, $p < 0.001$), as shown in Figure 2. Fractions of the main identified HO-PCBs of ΣHO-PCBs are shown in Figure 3. The PCBs from which the main metabolites may be formed are listed above each metabolite in Figure 3.

Figure 4 shows the correlation between one of the main HO-PCBs, 4-HO-CB146, and its potential precursor PCBs. The metabolite was significantly correlated ($p < 0.001$) with all possible precursor PCBs and was significantly correlated with many non-related PCBs (not shown).

Mean retinol concentrations were lowest in Nunavik samples and highest in southern Québec samples, but differences between the regions were not statistically significant (Table 3). No significant correlations were observed between concentrations of retinol and any individual HO-PCB or PCB congeners, ΣHO-PCBs, sum of all chlorinated phenolic compounds, or ΣPCBs.

Plasma concentrations of T_3, free T_4, TSH, and TBG were not significantly different between Lower North Shore and Nunavik samples (Table 3). Concentrations of main HO-PCBs and PCBs were not significantly correlated with thyroid hormone markers. In contrast, PCP concentrations were negatively correlated with T_3 ($r = -0.55$, $p = 0.01$), TBG ($r = -0.44$, $p = 0.05$), and free T_4 levels ($r = -0.51$, $p = 0.02$). Figure 5 shows the statistically significant inverse correlation ($r = -0.47$, $p = 0.01$) between free T_4 concentrations and log-transformed sum of all chlorinated phenolic compounds (sum of PCP and ΣHO-PCBs). The relationship was not improved using log-free T_4 and log-sum molar concentration of phenolic compounds. The sum of all chlorinated phenolic compounds was also negatively associated with T_3 concentrations ($r = -0.48$, $p = 0.03$). Concentrations of ΣPCBs and ΣHO-PCBs were both negatively correlated with TSH concentrations ($r = -0.46$, $p = 0.04$ and $r = -0.45$, $p = 0.04$, respectively).

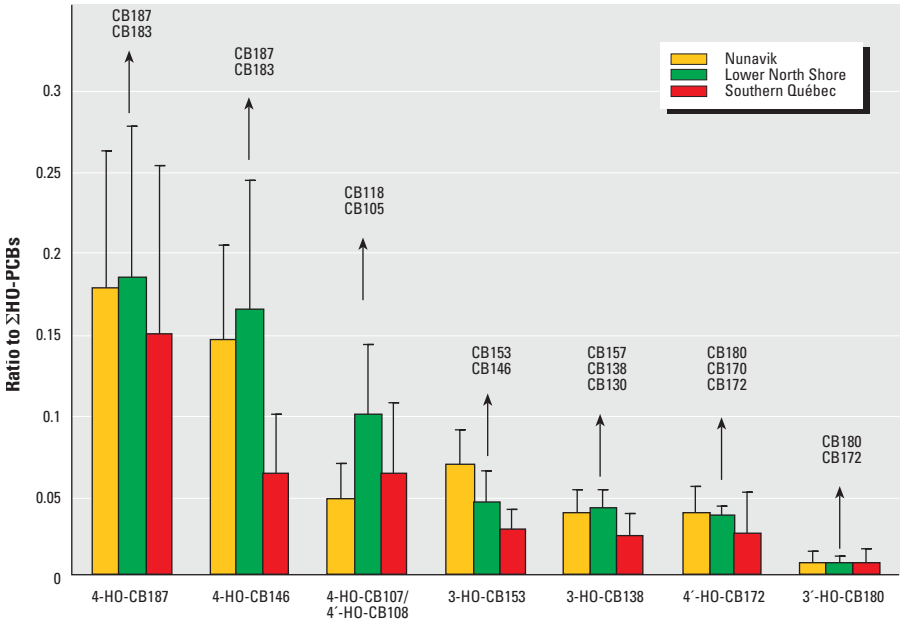

Figure 3. Concentrations of major HO-PCBs in umbilical cord plasma expressed as the mean fraction of ΣHO-PCBs. Error bars represent the SD. All potential precursor PCBs are given above each metabolite.

Discussion

Hydroxylated metabolites and other chlorinated phenolic compounds, to our knowledge, have never been examined in umbilical

cord plasma. We found that PCP was the most abundant phenolic compound in all three regions, representing an average of 78%, 66%, and 82% of the concentration of the sum of all quantitated chlorinated phenolic compounds in the Nunavik, Lower North Shore, and southern Québec groups, respectively. Mean PCP concentrations were similar among groups, and individual values ranged from 628 to 7,680 pg/g wet weight. We previously reported PCP as the dominant chlorinated phenolic compound in blood samples from Nunavik and southern Québec adults (*12*). Thus, PCP may supersede HO-PCBs as the chlorinated phenolic compound of highest concern in humans.

PCP and its salts have been used extensively as wood preservatives, biocides, and disinfectants (*32*). PCP use has been curtailed since the late 1970s and has been banned in some countries, such as Sweden (1977) and Germany (1987) (*32*). The use of PCP has been restricted in Canada since 1981. The main exposure to PCP for nonoccupationally exposed individuals is through the diet (*33*). Another significant source of PCP may occur through the metabolism of hexachlorobenzene (*34*). Plasma is the most important compartment for PCP storage. In dosed rats, 99% of PCP is bound to plasma proteins (*35*). In human volunteers, the percentage of PCP bound to plasma proteins was estimated to be 96% (*36*).

PCP can induce deleterious effects on several organs or tissues. Increased lymphocyte responses were noted in patients with high PCP blood levels (*37*). PCP can be metabolized to reactive quinone metabolites (*38*) with possible covalent binding to crude liver homogenates and isolated liver proteins *in vitro* (*39*). PCP has twice the affinity of T_4 to TTR (*10*) and has been shown to decrease circulating T_4 levels in rams exposed from conception (*40*). PCP also affects thyroid hormone metabolism by competitively inhibiting iodothyronine sulfation *in vitro* (*41*). In the present study, the sum of plasma concentrations of phenolic compounds, the major part being PCP, were negatively correlated to free T_4 and T_3 plasma levels. This suggests that PCP and perhaps other chlorinated phenolic compounds can alter thyroid hormone status in newborns, which in turn could lead to adverse neurodevelopmental effects in infants.

Another chlorinated phenolic compound recently identified by our laboratory in polar bear plasma, 4-HO-HpCS (*31*), was also found in all umbilical cord plasma samples analyzed. This is the first time this compound has been shown to be present in human plasma. The likely precursor for this compound is octachlorostyrene, an industrial byproduct. The fact that lower concentrations of 4-HO-HpCS were found in the southern Québec group than in the Nunavik and Lower North Shore groups suggests that the likely source of exposure is the consumption of species from the marine food chain. Sandau et al. (*31*) showed that this compound had an affinity similar to T_4 for binding to TTR, which is slightly less than PCP (*10*) and lower than most HO-PCBs that have been determined (*42*).

Concentrations of ΣHO-PCBs in umbilical plasma were highest in the Lower North Shore samples. More than 30 compounds were identified as HO-PCBs, of which 11 were positively identified with authentic standards. Three more HO-PCBs found in humans (*16*) were tentatively identified but could not be confirmed because no authentic standards were available. The main metabolite in 27 of the 30 samples was 4-HO-CB187. This compound was also the dominant metabolite in fish eaters from Sweden, Black-footed and Laysan albatross, and polar bear (*13,43,44*). Two possible parent PCBs can form 4-HO-CB187 through two different hydroxylation mechanisms. The first involves the direct insertion (*45*) of a hydroxyl group onto the para position of CB187. Direct insertion has been demonstrated to occur in *in vitro* metabolism studies of halobenzenes (*46*) and CB52 (*47*). CB187 is an abundant congener found in biota and accounted for a mean of 3.4% of the ΣPCBs in all the umbilical cord plasma samples. It is found as a small percentage (0.54%) in the Aroclor 1254 mixture, but is more abundant in Aroclor 1260 (5.4%) (*48*). The second mechanism of oxidation is the formation of a 3,4 (meta-para)-epoxide in CB183 followed by a 3,4 shift of chlorine to the meta position similar to the National Institutes of Health (NIH) shift of ^2H first described by Guroff et al. (*49*). Epoxide formation in the metabolism of PCBs has been demonstrated in *in vitro* studies (*50*) as well as *in vivo* studies (*17*) using CB77 as substrate. CB183 composed a mean of 0.8% of the ΣPCBs in the umbilical cord plasma and constitutes approximately 0.2% and 2.4% of Aroclor 1254 and 1260 mixtures, respectively (*48*). Interestingly, the major PCB metabolite in umbilical cord plasma, 4-HO-CB187, is formed from PCBs that make up a small percentage of the ΣPCBs in the samples.

The second most abundant metabolite in umbilical cord plasma was 4-HO-CB146. This metabolite can be formed by direct insertion onto CB146 or by NIH shift of

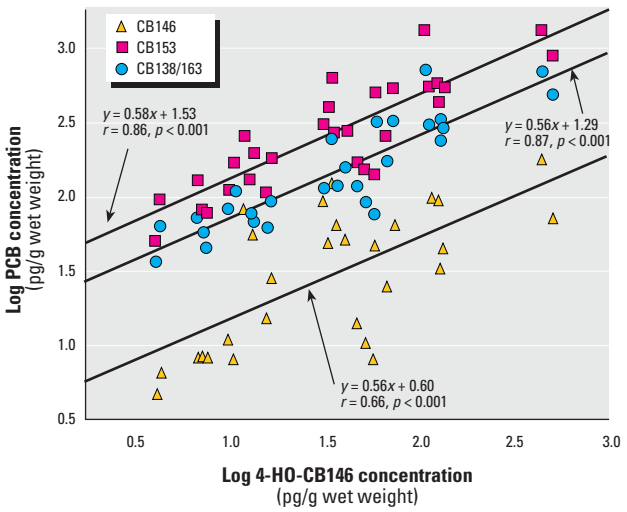

Figure 4. Relationship between log-transformed concentrations of precursor PCBs (CB146, 153, and 138) and the second most abundant metabolite in umbilical cord plasma, 4-HO-CB146. CB138 coelutes with CB163 and they were quantitated as a single peak.

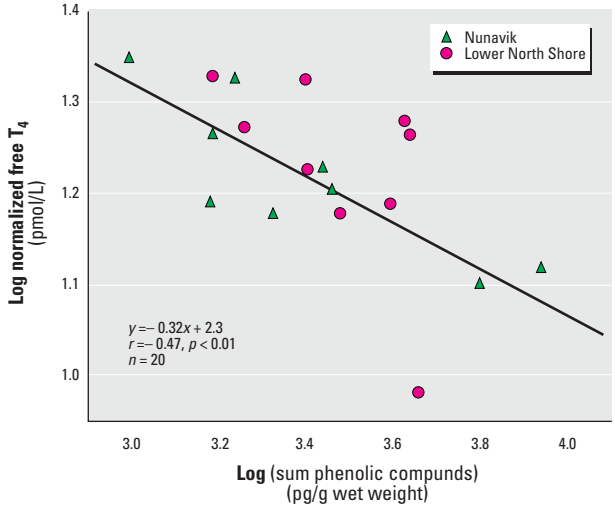

Figure 5. Relationship between log-transformed concentrations of free T_4 and sum of all chlorinated phenolic compounds (sum PCP and ΣHO-PCBs).

chlorine in the metabolism of CB138 or CB153. These three parent PCBs compose a large percentage of the ΣPCBs (between 11 and 47%) quantitated in all the samples. CB153 (mean 15% of ΣPCBs) and CB138 (mean 8.3% of ΣPCBs) are the two most abundant PCBs determined in the plasma samples and are major components in Aroclor mixtures (*48*). All three potential parent PCBs were significantly ($p < 0.001$) correlated with 4-HO-CB146 (Figure 4).

The third most abundant metabolite was 4-HO-CB107, which can be formed from CB107 (direct insertion), CB105 (NIH-Cl shift), or CB118 (NIH-Cl shift). Both CB105 and CB118 are major congeners in Aroclor 1254, composing 5.2% and 10.5% of the total (*48*). CB107 is a minor congener in Aroclor 1254 (0.6%), and it is rarely found in environmental samples, including these umbilical cord plasma samples. Concentrations of the potential parent PCBs CB105 (mean 1.3% of ΣPCBs) and CB118 (mean 4.8% of ΣPCBs) were significantly correlated ($r = 0.69$, $r = 0.81$, respectively; $p < 0.001$) with 4-HO-CB107 concentrations. In contrast to our study results, 4-HO-CB107 was previously found to be the main metabolite in adult Inuit whole blood, Latvian fish consumers, Baltic seals, white-tailed eagles, and rats dosed with Aroclor 1254 (*12,13,16,30*).

The relationship between metabolites and their potential precursor PCBs could not be resolved further using multiple-step regression analysis (forward or backward). Concentrations of major metabolites were highly correlated with all PCBs, even unrelated congeners. Therefore, it is not possible from the present data to determine which congeners are the precursors of the metabolites—that is, the relative importance of NIH chlorine shift to direct insertion.

Hydroxylated PCB patterns vary among individuals (*12,13*). This variation can be caused by selective retention or selective formation of metabolites or by differences in PCB exposure. The retention of specific HO-PCBs is probably similar for all humans. The main structural requirement for retention is the capability to bind to TTR (*9*). This requirement is thought to involve a hydroxyl group with adjacent chlorines (*42*). The hydroxyl group is often in the para position of the biphenyl ring, but not exclusively, because meta-substituted metabolites are also found in plasma. Humans have varying concentrations of TTR in plasma, and some genetic abnormalities are known (*51*). Generally, concentrations are in excess molar concentration to HO-PCBs (*12*). Thus, the main determinant of the pattern of HO-PCBs in blood is likely the formation of metabolites from the parent PCB congeners.

It was interesting to note that the geometric mean ratio of ΣHO-PCBs to ΣPCBs concentrations was similar (~ 0.2) among regions. The ratio was twice that found in a previous study involving whole blood of Canadian Inuit (0.11) (*12*). Because the relationship of the log-transformed concentrations in umbilical cord plasma had a slope of between 0.5 and 0.6 (Figures 2 and 4), the ratio of metabolites to PCBs decreased with increasing PCB concentrations. The range was from approximately 0.4 at low PCB concentrations (500 pg/g; Figure 2) to approximately 0.1 at high PCB concentrations (5,000 pg/g; Figure 2), similar to that found in adult whole blood. There was no apparent effect of PCB concentration on the ratio of metabolites to PCBs in adult whole blood (*15*). The generally higher ratio in umbilical cord plasma samples may reflect the difference in composition of fetal and adult blood. For example, umbilical cord plasma has approximately half the lipid content and less transthyretin than adult plasma (*52*). It has been shown previously that PCBs are most concentrated in plasma lipoproteins (*53*). Another possible explanation for the differences in the metabolite/PCB ratio in adult and fetal blood could involve enhanced placental transfer of HO-PCBs from the mother. The transfer of PCB metabolites from dosed mice to the fetus was tested by Sinjari et al. (*20*). They showed that 4′-HO-CB79 concentrations in fetal plasma were twice that of the maternal plasma, 24 hr after exposure, indicating enhanced transport of the metabolite, which likely occurs through binding to TTR.

When the individual chemical residue data were compared to thyroid hormone markers, only PCP concentration was significantly related to T_3, free T_4, and TBG concentrations. PCP has twice the affinity of T_4 to TTR (*10*) and can affect thyroid hormone concentrations in rats (*54*). PCP has also been shown to affect thyroid hormone metabolism by competitively inhibiting iodothyronine sulfation *in vitro* (*41*). When concentrations of all phenolic compounds were summed and correlated with the thyroid hormone markers, only T_3 and free T_4 concentrations were negatively associated, and the significance of the regression increased. The negative association between free T_4 and the sum of all phenolic compounds is in agreement with the theory that HO-PCBs and other chlorinated phenolic compounds disrupt thyroid hormone transport through the common mechanism of binding to TTR. In addition to binding to TTR, halogenated phenolic compounds may disrupt thyroid hormone metabolism (*14,41*).

Morse et al. (*55*) found that both maternal and neonatal rats showed decreased total and free T_4 levels with exposure to CB169 and/or CB77 in a dose-dependent manner. They concluded that fetal T_4 levels were affected by both reduced transplacental delivery of T_4 and increased T_4 metabolism by the induced glucuronyltransferase enzymes. Darnerud et al. (*56*) also demonstrated fetal reduction in total T_4 and free T_4 when pregnant mice were dosed with CB77. Furthermore, Dutch infants showed decreased free and total T_4 levels with increased PCB/dioxinlike compound exposure (*23*). Thus, many studies indicate that T_4 concentrations can be decreased by exposure to PCBs, and this study supports the theory that HO-PCBs, and perhaps other halogenated phenolic compounds, may be partly responsible for this decrease.

TTR has been shown to be important in T_4 transport in cerebral spinal fluid (*7*). If chlorinated phenolic compounds can significantly alter plasma levels of TTR-bound T_4, this may lead to brain thyroid hormone deficiencies *in utero*, possibly affecting brain development (*57*). TTR is also important in thyroid hormone transport across the placental barrier (*58*). Maternal sources of thyroid hormones are thought to influence fetal brain development (*59*). The binding of metabolites to TTR may also improve transport of halogenated phenolic compounds across the placenta, as has been shown in mice (*19*). Thus, phenolic compounds may be able to disrupt maternal sources of thyroid hormones, penetrate into fetal circulation, and disrupt local thyroid hormone supply in the developing fetus. The potential of PCP and HO-PCBs to disrupt thyroid hormone homeostasis in the developing fetus warrants further investigation to confirm the effects observed in the present study. A study is currently underway that will examine the relationship between halogenated phenolic compounds, thyroid hormones, and retinol concentrations in newborns from a larger cohort.

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